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MINI REVIEW ARTICLE

Solid-Phase Synthesis of Asymmetric Cyanine Dyes

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Abstract: Cyanine dyes (CD) are a functional class of organic molecules used in several applications ranging from photography to bioimaging. CDs most well-known features resides on high molar extinction coefficients up to 10^5 L $mol^{-1}cm^{-1}$ and on the absorption spectra, ranging from 500 to 1000 nm, which can be fine-tuned both by extending the length of the central methylene bridge or by modulating the terminal heterocycles. In the last decades, new synthetic methodologies, namely microwave-assisted and the solid-phase procedure, have been developed to overcome the limitation of the classical synthetic protocols. While microwave approach usually reduces the exposure time of the reagents and products to thermal degradation, the solid-phase methodology allows easier synthetic protocols which is translated in higher yields and simpler products purification. In the present review a comprehensive analysis of the solid-phase methods for the synthesis of asymmetrical CDs is discussed, with a critical evaluation of the difference among the currently available solid-state approaches.

Keywords: Cyanine Dyes, Asymmetric Dyes, Solid State synthesis, Resins, Solvent-free, User-friendly method

1. INTRODUCTION

Cyanine dyes (CD) are a class of organic functional dyes characterized by a chemical structure in which two nitrogen atoms are linked through a single or multiple methine group to form a delocalized system containing an odd number of atoms (Figure 1). The overall structure is fully conjugated with the π electrons delocalized along the whole molecular backbone while the positive charge is located on the nitrogen making the CDs depictable as a resonance hybrid of two structures [1-4]. By a structural point of view, the conjugated carbon bridge plays a key role on the photophysical properties of CDs leading to a bathochromic shift of around 100 nm upon each addition of a methylene unit (Figure 2) [4-5]. Cyanines can thus cover a large part of the visible spectra by simply tuning the length of the polymethine bridge. This peculiar features along with the remarkable molar extinction coefficients ≈1.5-3 x 10^5 L $mol^{-1}cm^{-1}$ made CDs one of the most explored class of chromophores for various applications like photography, sensors, fluorescence imaging, data storage, nucleic acid labelling, medicine, dyes-sensitized solar cells [5-24]. CDs are generally named according to the length of the chain between the two nitrogen atoms.

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The dyes with one, three, five and seven methine units are called mono-, tri-, penta- and heptamethine respectively [4].

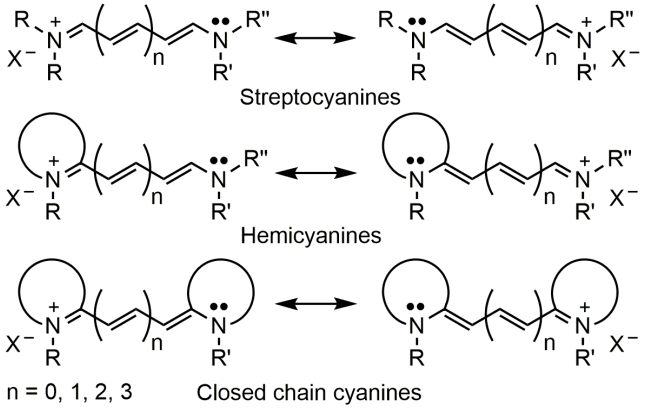

Figure 1. Schematic representation of the different CDs as a function of the number of methine units and terminal groups.

However, an additional classification is commonly reported based on the chemical structure of the groups bearing the nitrogen atoms (Figure 1). Cyanines without any terminal heterocyclic groups are named streptocyanines, those with only one terminal heterocycle are hemicyanines and, those with two heterocycles at the chain edges are called closed chain cyanines [25-26].

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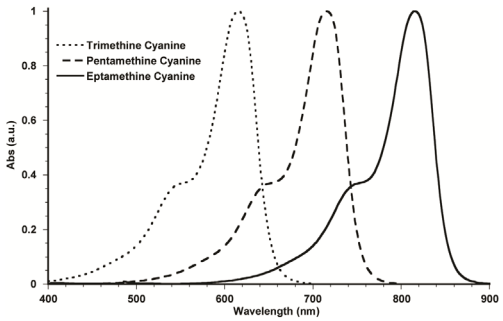

Figure 2. Bathochromic shift of the absorption spectra of symmetric CDs by extending the length of the methine bridge from tri-, to penta- and finally hepta- CDs.

In the latter family, a further subclassification is used by the nature of the heterocyclic structures such as indole, benzoindole, benzoxazole, benzothiazole, benzoselenazole, quinaldine and lepidine (Figure 3). The nature of the two terminal groups not only classifies the CDs but also affects the electronic behaviour of the overall dye. When the terminal moieties on the edges of the polymethine chain are identical, the dye has an even electron density distribution and these cyanines are called symmetrical. On the other hand, when the termini are different one to another the CDs are defined asymmetrical [27]. In both cases, for synthetic purposes, the heterocyclic precursors must carry a methyl group in α o γ position to nitrogen atom whose quaternization increases methyl acidity. The common synthetic strategy to prepare symmetrical CDs, except for the monomethine cyanines, resides on the condensation of two equivalents of quaternary ammonium salts with the polymethine chain linker in a one-pot procedure [4,28].

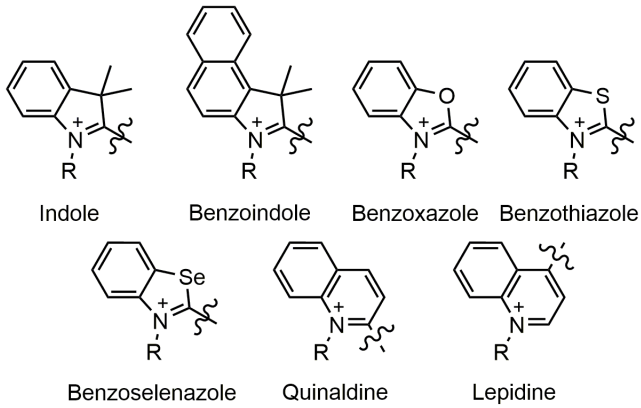

Figure 3. Schematic representation of the heterocyclic moieties used to synthetize CDs.

The general reaction mechanism implies several steps starting with (i) a base (e.g. triethylamine, pyridine, acetate salts) promoted deprotonation at the α or γ methyl group of the quaternary ammonium salt, leading to the formation of a nucleophilic methylene unit (Figure 4). The active methylene (ii) attacks the dianilide-based linker forming a new carbon-carbon bond and (iii) promoting an electronic reorganization which releases a secondary amine as a leaving group. A final proton extraction (iv), by a base, leads to the formation of the hemicyanine with a full conjugated structure. The hemicyanine is then subjected to the same reaction pathway (v and vi) with another equivalent of the quaternary salt to provide the final symmetrical dye.

According to the desired final CDs structure, various precursors can be used such as orthoesters for trimethine bridge, malondialdehydes and their dianilide-based derivatives for pentamethine scaffolds, glutaconaldehyde dianilide and Vilsmeier-Haack reagent for heptamethine dyes. This synthetic approach can also be applied to the synthesis of unsymmetrical dyes. Nevertheless, due to the presence of the concurrent formation of the symmetrical analogs, the asymmetrical dyes are generally formed in low yield and upon challenging purification. To overcome these limitations arising from the one-pot synthetic procedure, unsymmetrical CDs can be accessed by different methodology based on the polymethine bridge length [29].

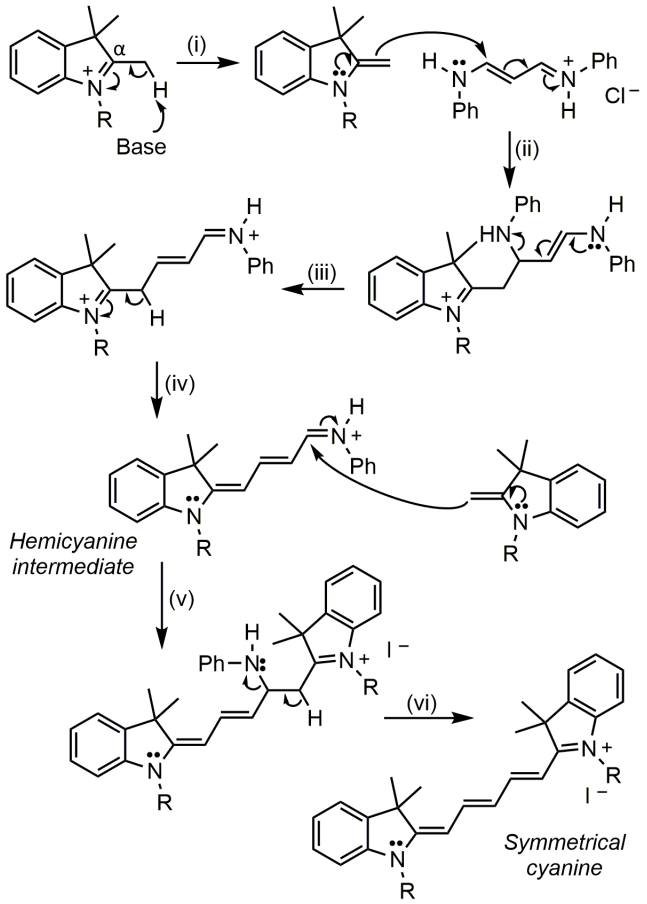

Figure 4. General reaction mechanism to form symmetrical CDs. (i) Deprotonation of the α methyl group of the quaternary ammonium salt; (ii) attack of the dianilide-based linker by the activated methylene; (iii) electronic reorganization and release of the secondary amine; (iv) formation of the hemicyanine scaffold; (v) attack of the hemicyanine by another activated quaternary salt; (vi) electronic reorganization and elimination of the secondary amine to yield the symmetrical cyanine.

The monomethine CDs can be easily obtained by a single step procedure based on the "thioether method" while tri-, penta- and heptamethine dyes can be accessed by multistep methods, namely the "aldehyde method" and the "hemicyanine method" (Figure 5a and 5b). The "aldehyde method" involves the use of a methine source and of a quaternary salt to yield an aldehyde derivative which reacts in a second step with a different quaternary salt obtaining the final unsymmetrical structure. The appropriate choice of the methine source addresses the length of the methine bridge.

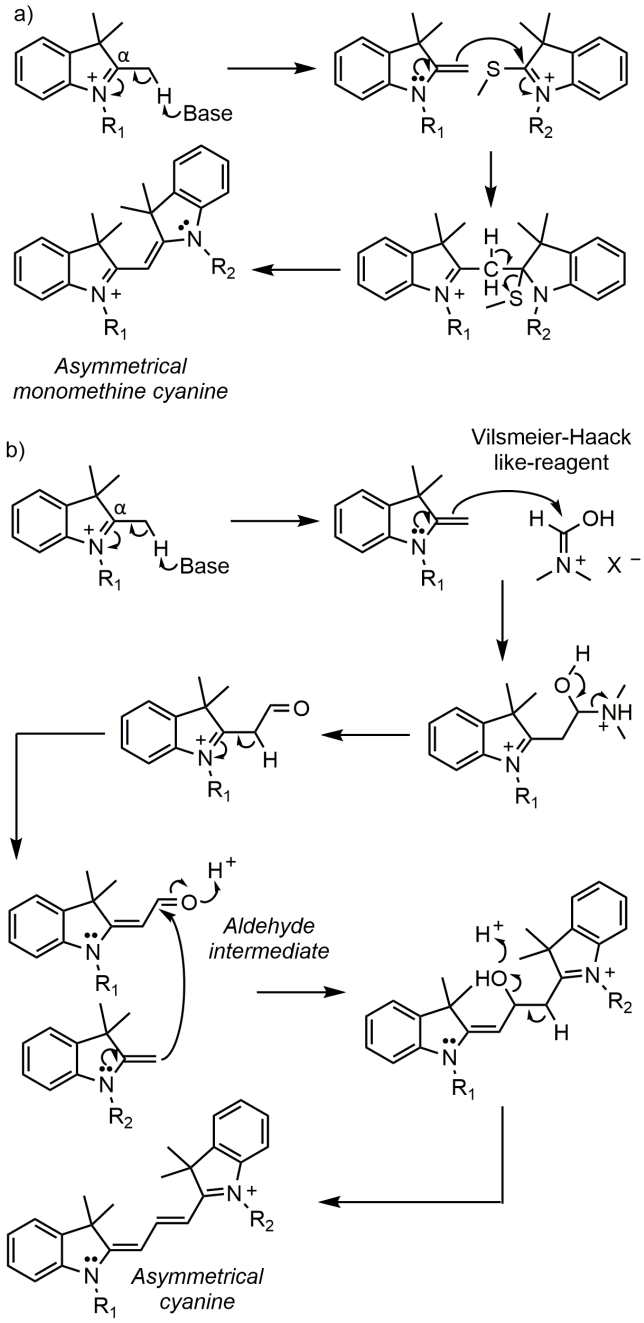

Figure 5. a) General mechanism of "thioether method". b) General mechanism of "aldehyde method".

$POCl_3$ and *N*,*N*-dimethylformamide provide trimethine dyes, pentamethine CDs are prepared with tetraethoxypropane while 1-(dimethylamino)-5-formyl-1,3-butadiene is usually employed in the preparation of the heptamethines analogs. The "hemicyanine method" resembles the procedure described for the preparation of the symmetrical dyes but differs on the milder reaction conditions and ratio between the reagents. The first step involves the formation of the hemicyanine structure, while the second one provides the final asymmetrical cyanine by reaction with the nucleophilic methylene of another quaternary salt. Usually the hemicyanine intermediate requires to be activated, for example by *N*-acylation to ensure an efficient reaction with the second quaternary salt. Nevertheless, during the first step, the formation of the undesired symmetrical dye cannot be completely avoided even by performing the coupling with an excess of polymethine linker. Although the above-mentioned approaches ensure the synthesis of the desired asymmetrical CDs, it is worth noting that multistep procedures and non-trivial purifications are required [30].

Solid-state synthesis applying resin-based methodology or solvent-free procedures have been introduced in the last decades to overcome these limitations and providing CDs with higher yield and easier to be purified. In this short review, we report a comprehensive analysis of the nowadays available different solid-phase methods applied in the synthesis of asymmetrical cyanine dyes.

2. SOLID-PHASE SYNTHESIS OF ASYMMETRICAL CYANINES

Solid-phase organic synthesis and combinatorial chemistry are well-known approaches, involving the use of resins, for the synthesis of peptides and drugs [31, 32]. However, in the last years they have found a broader application in the synthesis of different type of dyes such as styryl dyes, BODIPYs, phthalocyanines and cyanines [33-42]. In particular, the use of solid-phase synthesis has become a powerful technique to cleanly synthesize asymmetrical CDs with high degree of purity and without tricky and expensive purification protocols. Two main methodologies have been developed based on the solid-phase approach, the "catch-and-release" method and the "immobilized imidate" method. The former involves three distinct steps: (i) the synthesis of hemicyanine scaffold, (ii) the catch of the hemicyanine onto a resin, (iii) the final formation and later cleavage of the unsymmetrical cyanine dye from the resin (Figure 6) [38].

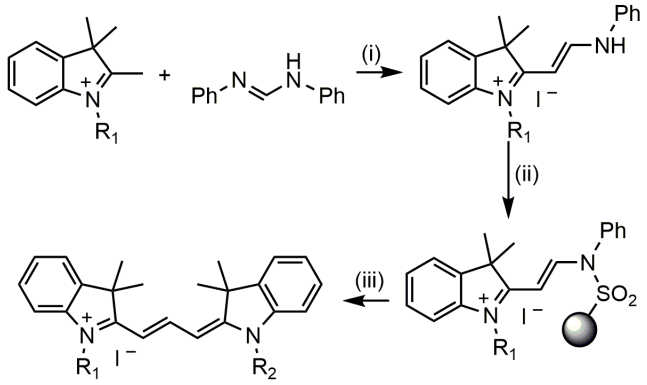

Figure 6. "Catch-and-release" solid-phase synthesis. (i) Synthesis of the hemicyanine by classical in solution methodology, (ii) immobilization of hemicyanine onto resin, (iii) formation and later cleavage of the unsymmetrical cyanine dye from the resin.

The immobilization of the hemicyanine onto the resin has the dual function of activating the hemicyanine and of facilitating the removal of the major byproducts. The hemicyanine intermediates are relatively unreactive at room temperature but can be activated by functionalizing the nitrogen with an electron-withdrawing group like by acetylation or sulfonylation [43, 44]. In a similar manner, the reaction of the hemicyanine with a sulfonyl chloride polymer-bound resin not only activates the substrate but also allows the removal of the undesired byproducts, mainly the symmetrical CD, by simple washing the resin with compatible solvents. Finally, the reaction of bound-

hemicyanine with another heterocyclic carbon nucleophile leads the concurrent formation of the desired asymmetrical CD and the release from the resin. Nevertheless, it must be pointed out that the use of a substoichiometric amount of the second heterocyclic nucleophile is crucial in the cleavage step to minimize the contamination of the final product. The pure product can be easily obtained by the separation of resin by filtration. The "catch-and-release" methods allows an easier purification of the final dye in comparison to the classical synthesis in solution but still requires the synthesis and purification of the hemicyanine scaffold which, usually, is not so straightforward.

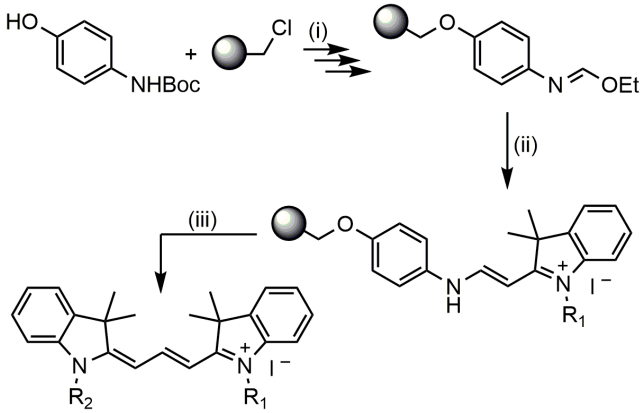

Figure 7. "Immobilized imidate" solid-phase synthesis. (i) Construction of resin-bounded imidate, (ii) Synthesis of immobilized hemicyanine, (iii) formation and later cleavage of the unsymmetrical cyanine dye from the resin.

The "immobilized imidate" method was developed to overcome the above-mentioned limitation of the "catch-and-release" procedure. The approach is based on three distinct steps: (i) the construction of resin-bounded imidate, (ii) the synthesis of a polymer-bounded hemicyanine scaffold, (iii) the final formation and the later cleavage of the unsymmetrical cyanine dye from the resin (Figure 7) [39]. The "immobilized imidate" method allows an easier purification of hemicyanine due to the immobilization onto resin since the first synthetic step in comparison to the "catch-and-release" strategy.

2.1 Solid-phase synthesis of asymmetrical monomethine cyanines

Tri-, penta- and heptamethine CDs can be yielded, as previously described, either by the "aldehyde" or the "hemicyanine" methods, while asymmetrical monomethine cyanines are synthetized by a slightly different approach, the "thioether method". This difference in synthetic procedure is even found in solid-phase methodology. Monomethine cyanines **1** and **2** were the first ever asymmetrical CDs synthetized by a solid-phase approach (Figure 8a) [37]. The dye **2**, better known as Thiazole Orange (TO), has found large application in chemical biology to stain nucleic acids and proteins due to a remarkable fluorescence [46]. The first synthetic step in the preparation of **1** and **2** consisted on the attachment of either the picoline- **3** or the lepidine-based **4** precursors to the Rink amide MBHA polystyrene (PS) resin through the carboxylic function on the pendant chain (Figure 8b). The quaternary salts **3** and **4** were coupled to the resin with a four-fold molar excess using the conventional reagents hexafluorophosphate benzotriazole tetramethyl uronium (HBTU) and *N*,*N*-diisopropylethylamine (DIEA) in DMF/pyridine (1:1). The obtained coupled-resins intermediates **5** and **6** were subjected to react with the benzothiazole intermediate in four-fold molar excess in the presence of triethylamine (TEA) in dichloromethane (DCM) yielding the resin-coupled final product **7** and **8**. Finally, the desired compounds **1** and **2** were cleaved from the resin by a treatment by a 95% trifluoroacetic acid aqueous solution. Monomethine cyanines **1** and **2** were obtained in almost quantitative yield and with high grade of purity through few simple solvent-rinsing [37].

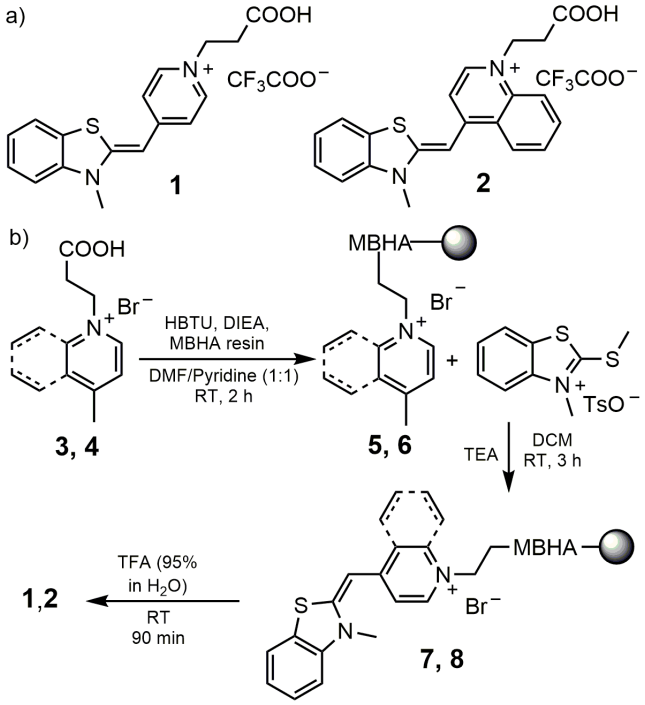

Figure 8. a) Asymmetrical monomethine **1** and **2** structures; b) Solid-phase synthesis of **1** and **2** with Rink amide MBHA PS resin.

Several other modified TO-based monomethine CDs **9**-**19** were synthetized by solid-phase method different from the one described for the preparation of compounds **1** and **2** (Figure 9a) [40]. While in the previous method, the resin was functionalized with a quaternary ammonium salts, the procedure to synthetize **9**-**19** involves: (i) the immobilization of a benzothiazole intermediate **20**-**24** onto Merrifield resin providing **25**-**29**, (ii) the quaternarization of benzothiazole's nitrogen leading to the precursors **30**-**34** and finally (iii) the formation and later cleavage of the TO cyanine dyes from the resin (Figure 9b). Monomethine cyanines **9**-**19** were obtained with yield higher 90% with high grade of purity through few simple solvent-rinsing [40]. This approach closely resembles the above described "immobilized imidate" even if it does not involve directly an imidate intermediate. A particular attention has been dedicated to exploring the immobilization of the initial benzothiazoles **20**-**24** onto the Merrifield resin by evaluating the effect of different solvents, temperature, reaction time and substituents on the heterocycle. A good balance among those solvents' parameters have been shown to provide higher reaction conversion, while single high value of one of the solvent physical parameters is not enough to improve the reaction yield (Table 1).

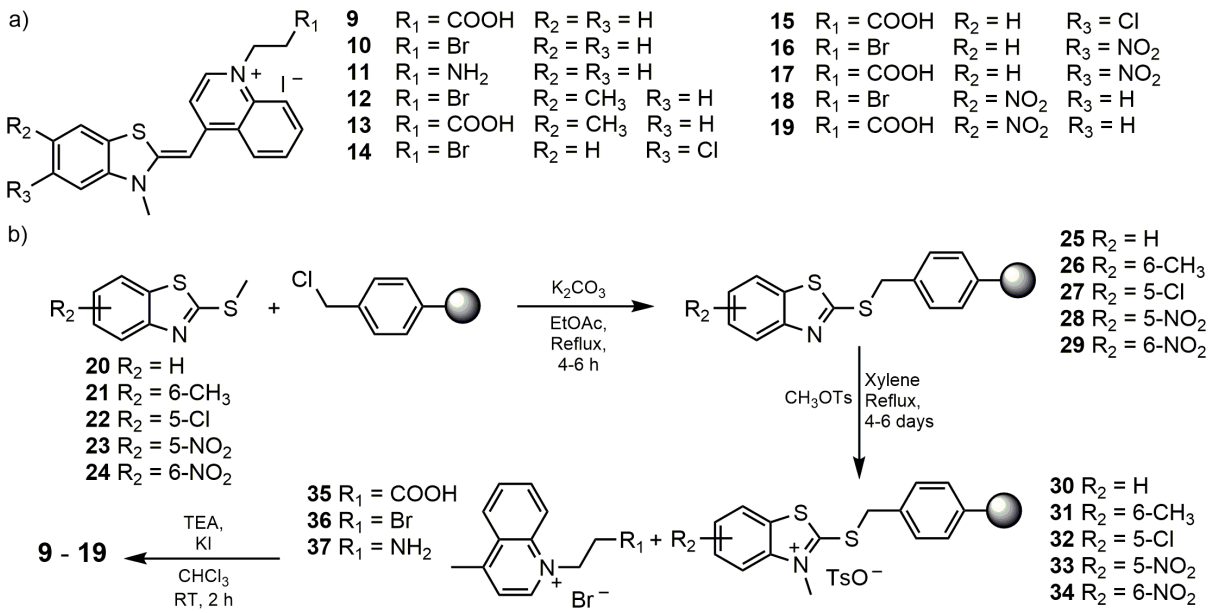

Figure 9. a) Structure of the monomethine CDs **9**-**19** synthetized with Merrifield resin; b) Solid-phase synthesis of **9**-**19** with Merrifield resin by immobilization of benzothiazole derivatives, quaternarization of the immobilized-benzothiazole's nitrogen, formation and later cleavage of the TO cyanine dyes from the resin.

Ethyl acetate ensure the best conversion rate due to the good compromise among the above-mentioned parameters. Moreover, acetone and toluene have shown good results but the low boiling point of the former, and the low dielectric constant and dipole moment for the latter, negatively affect the conversion rate.

Table 1. Experimental conversion in different solvents

Solvent	Boiling point (°C)	Dielectric constant (25 °C)	Dipole moment (D)	Conversion rate (%)[a]
Toluene	110	2.40	1.33	85
Ethyl acetate	77	6.02	1.89	89
Chloroform	61	4.81	1.15	76
Acetone	56	20.70	2.85	80

[a] Data acquired using 2-mercaptobenzothiazole. Reaction time: 4 h.

Temperature and time also affect the overall reaction conversion in a remarkable way. Too low temperature has shown to hamper the product formation, while four hours has been found as sufficient time to maximize the product formation (Table 2). Finally, also the effect of the substituent on the benzothiazole ring has been investigated, suggesting that strong electron-withdrawing groups (e.g. NO_2) can significantly lower the conversion rate without regards to the position on the benzothiazole scaffold.

Table 2. Reaction conversion screening

	Temperature (°C)		
	16	**55**	**reflux**
Conversion rate (%) of 20	10[a]	80[a]	89[a]
	Time (h)		
	1	**2**	**4**	**6**
Conversion rate (%) of 20	43[b]	70[b]	89[b]	89[b]
	Substrate				
	20	**21**	**22**	**23**	**24**
Conversion rate (%)	70[b]	66[c]	82[c]	38[c]	43[c]

[a] Reaction time: 4 h. Solvent: ethyl acetate.
[b] Solvent: ethyl acetate. Temperature: reflux.
[c] Reaction time: 2 h Solvent: ethyl acetate. Temperature: reflux.

2.2 Solid-phase synthesis of asymmetrical trimethine cyanines

The asymmetrical trimethine CDs are the family of cyanines that has been most explored using solid-phase synthesis. Up to date just two asymmetrical trimethine cyanine, **38** and **39** have been synthetized with the method described for the monomethine scaffold [37]. Most of the reported CDs **40**-**53** by solid-phased synthesis were obtained either by the "catch-and-release" method or the "immobilized imidate" method (Figure 10).

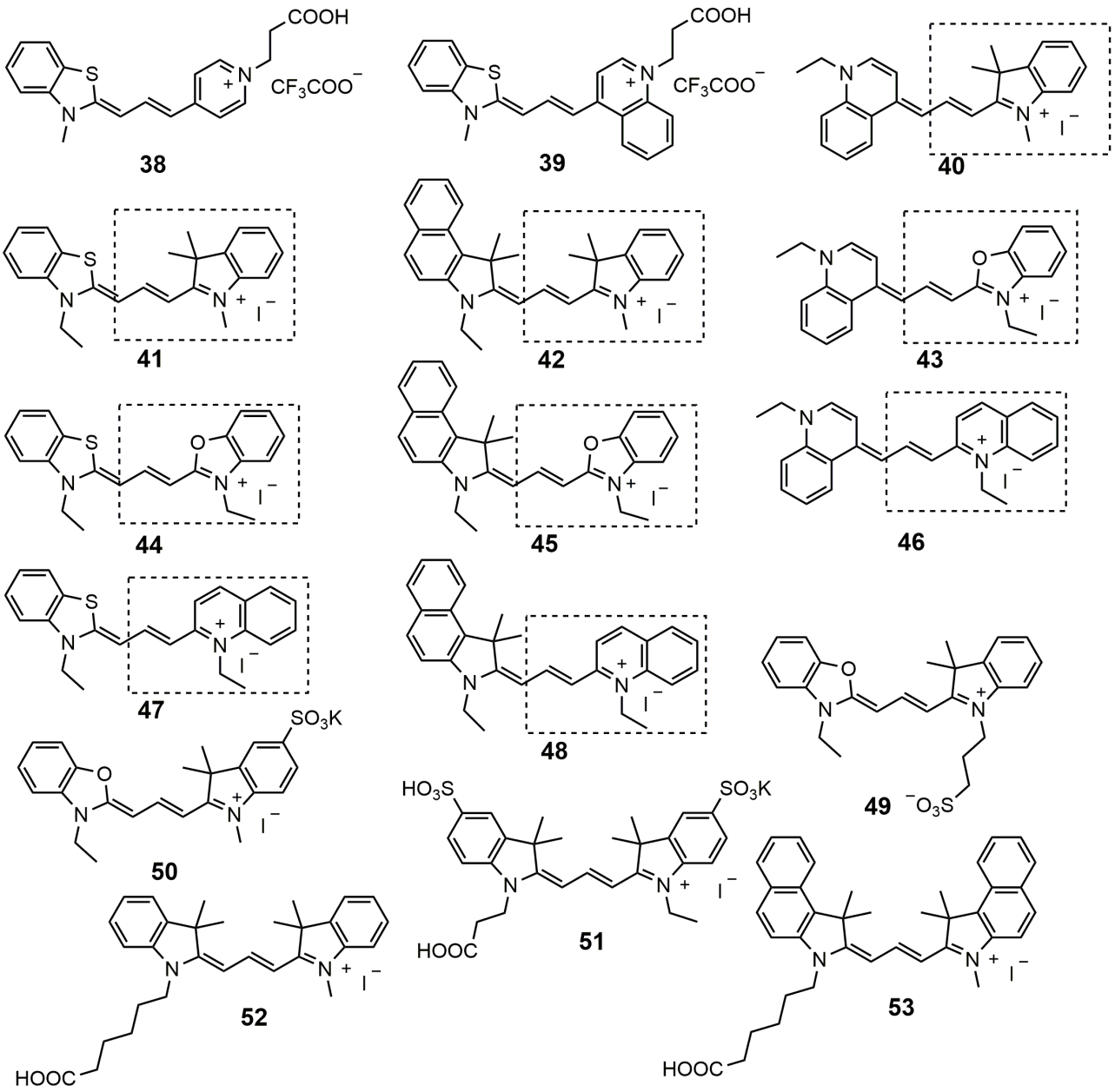

Figure 10. Asymmetrical trimethine cyanine dyes synthetized by solid-phase method; the moiety linked to the resin is indicated by the dashed box.

A systematic comparison of the solid-state synthesis using the two different methodology has been explored by varying both the resin-attached cyanine intermediate and the coupling ammonium quaternary salt yielding the dyes **40**-**48** (Table 3) [38, 39]. At first, the "catch and release" method has been explored by preparing three hemicyanine intermediates starting from the ammonium quaternary salts of an indole, a benzoxazole and a quinaldine scaffolds [38]. The purified hemicyanine were used in three-fold excess to reach with the sulfonyl chloride moieties on a resin in presence of DIEA, DCM at room temperature for four hours. Finally, the resin-attached hemicyanines were coupled with three different ammonium quaternary salts based on a benzoindole, a benzothiazole and a lepidine core respectively to provide the CDs **40**, **41** and **43**-**47** with yields ranging from 19 to 79%. The formation and cleavage reactions were performed using a 0.3 equivalents of quaternary ammonium salt, in binary solvent mixture of DIEA pyridine 1:9 for 30 minutes at room temperature. Interestingly, products **42** and **48** were not formed most likely due to the less reactivity of the benzoindole-based quaternary salt in comparison to the benzothiazole- and lepidine-based analogs. However, it was also reported that the low reactivity of benzoindole scaffold can be improved using a stronger base such as NaH [38]. Looking at the immobilized hemicyanine, the systematic investigation has highlighted that the indole-based core has led to higher product yields in comparison to the benzoxazole and quinaldine moieties while an immobilized benzoxazole scaffold has provided the higher values of compounds purity [38]. The "immobilized imidate" strategy, using the previously described combinations between the linked-intermediates and the quaternary ammonium salts, has generally provided CDs **40**-**48** in higher yields and better purities including, in this case, the cyanines **42** and **48** [39].

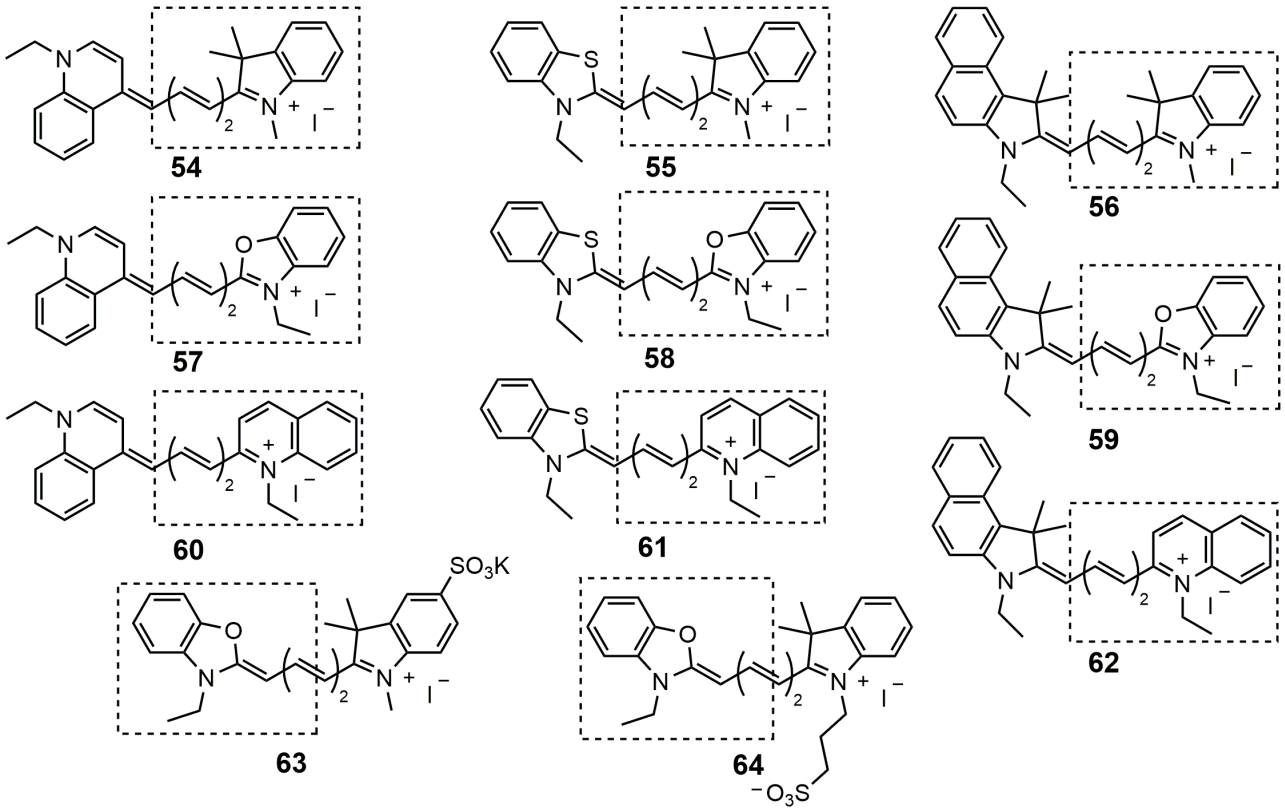

Figure 11. Asymmetrical pentamethine cyanine dyes synthetized by solid-phase method; the moiety linked to the resin is indicated by the dashed box.

Table 3. Reaction yield and product purity comparison

	"Catch-and-release"		"Immobilized imidate"	
Cpd	**Purity**[a]	**Yield**[b]	**Purity**[a]	**Yield**[b]
40	89%	79%	> 95%	19%
41	91%	60%	> 95%	26%
42	< 1%	-	89%	54%
43	> 95%	43%	> 95%	58%
44	> 95%	23%	> 95%	30%
45	50%	32%[c]	> 95%	76%
46	93%	42%	> 95%	70%
47	84%	19%	> 95%	23%
48	< 1%	-	50%	53%

[a] Determined by HPLC with ELSD quantitation.
[b] Crude yield (based on amount of heterocycle in cleavage step).
[c] Improved yields and purities obtained by prior deprotonation with NaH followed by reaction with immobilized hemicyanine.

In addition, water soluble trimethine CDs **49** and **50** were prepared with the same methodology with good yield of 73 and 59% respectively. It is worth nothing that the sulfonated heterocycles did not react well in the loading reaction with the imidate, while were providing good results as nucleophiles in the dye formation and cleavage step. Similar hydrophilic dyes, bearing sulfonic and/or carboxylic groups, based on an indole core **51**, **52** and a benzoindole scaffold **53** have been prepared with the same methodology [41]. The overall reported yields of 94, 68 and 49% for **51**, **52**, and **53** respectively have confirmed the lower reactivity of the benzoindole-based quaternary salts.

2.3 Solid-phase synthesis of asymmetrical penta- and heptamethine cyanines

The asymmetrical penta- and heptamethine cyanine dyes have been less explored by the solid-phase synthesis in comparison to the trimethine analogs. The "catch and release" method has been explored for the preparation of the pentamethine CDs but has shown limitation due to a competitive hydrolysis process [39]. On the other hand, the "immobilized imidate" approach has allowed the preparation of various dyes **54**-**64** in moderate to good yields (Figure 11) [39,41].

Table 4. Reaction yield and product purity penta CDs

	"Immobilized imidate"				
Cpd	**Purity**[a]	**Yield**[b]	**Cpd**	**Purity**[a]	**Yield**[b]
54	91%	60%	**60**	66%[c]	-
55	> 95%	92%	**61**	50%[c]	-
56	> 95%	96%	**62**	76%	57%
57	> 95%	12%	**63**	> 95%	64%
58	> 95%	72%	**64**	> 95%	86%
59	92%	46%			

[a] Determined by HPLC with ELSD quantitation.
[b] Crude yield (based on amount of heterocycle in cleavage step).
[c] Dyes not clearly separated by HPLC; purities estimated by ^{1}H-NMR integration.

The elongation of the polymethine linker itself has been shown to have unpredictable effect on the reaction yields for **54**-**64** when compared to the trimethine products (Table 4) [39]. For resin-linked substrate based on the indole core **54**-**56** the yield in the final product is remarkably higher in comparison to the analogs **40**-**42** while the product purity is

not much affected. Benzoxazole-based **57**-**59** were characterized by comparable degree of purity but lower product yield except for **58**. Finally, the pentamethine CDs prepared by quinaldine-immobilized substrates **62**-**64** were affected by lower degree of purity for **62** and **63** which hampered the isolation of the desired dyes.

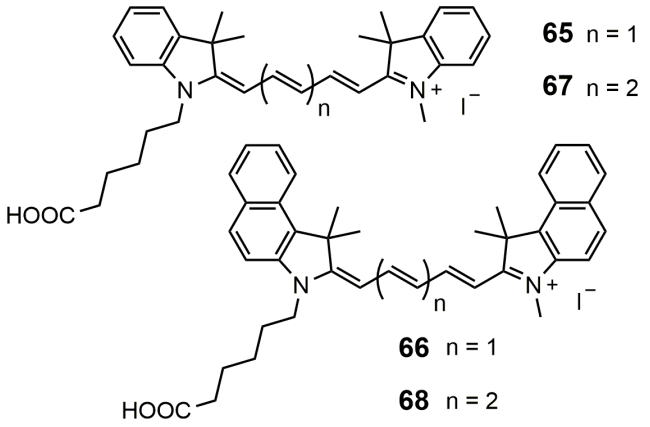

Figure 12. Asymmetrical penta- and heptamethine cyanine dyes synthetized by solid-phase method.

Extending the oligomethine bridge of **52** and **53** has provided the pentamethine CDs analogs **65** and **66** with a remarkable improve in the yields from 68 and 49% to 84 and 92% respectively (Figure 12) [41]. A further extension of the conjugated bridge allowed the solid-phase preparation of heptamethine derivatives **67** and **68** with a minimal to consistent drop in the product yields to 86 and 51% respectively.

2.4 Water-soluble cyanine dyes using poly(ethylene glycol) as a soluble support

The solid-phase synthesis of CDs has reduced the amount of chemical waste (e.g. solvent) needed for the nontrivial purifications required by the classical in solution protocols. Nevertheless, the above-described procedures in solid state still required the use of organic solvents to wet the resins and allow the reactions at the interface with the quaternary salts. An alternative approach using poly(ethylene glycol) (PEG) as a water-soluble support has been explored to synthesize unsymmetrical cyanines **69** and **70** analogs of the water-soluble **51** in an more environment friendly way (Figure 13) [46].

Even though this synthesis is not strictly conducted at the solid state, the comparison with the above strategies still provides useful information. The PEG resin is at first functionalized with para-aminobenzoic acid **71** to lead a free amine functionalized PEG **72** which is converted into **73** or **74** by reaction with the triethyl orthoformate or the 1,1,3,3-tetramethoxypropane. Further reaction with the appropriate quaternarized nucleophile resulted in the formation of the di- or tetra-methine bound hemicyanines **75** and **76**. The final coupling with a second quaternary ammonium salts provided the desired asymmetrical CDs **77** and **78**. The two dyes were isolated without the need of tricky purification in overall yields upon three steps of 22 and 24% respectively.

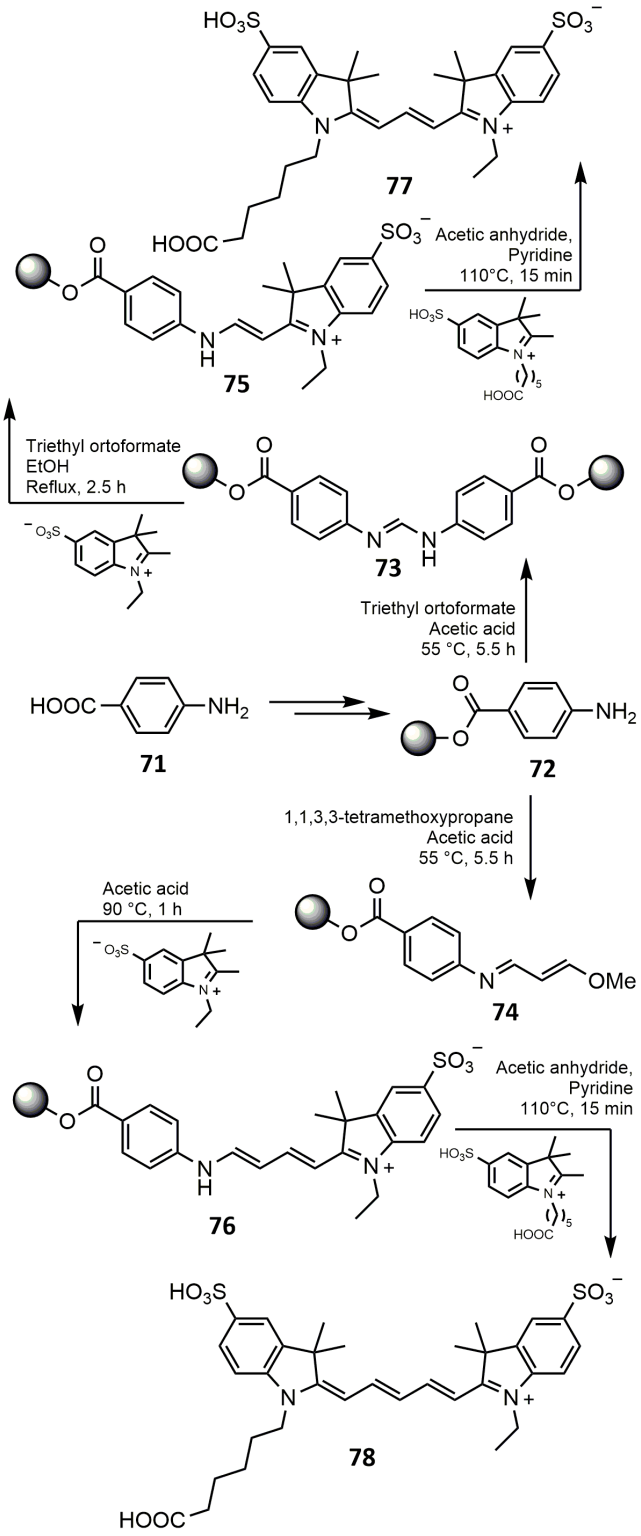

Figure 13. PEG-supported synthesis of trimethine **77** and pentamethine **78** cyanine dyes.

3. SOLVENT-FREE SYNTHESIS OF ASYMMETRICAL CYANINES

In the last two decades, alternative solid-state synthesis of cyanines have been explored even without the use of a resin support. Solvent-free reactions avoid the use of large amount of organic solvents, reducing the pollution and the costs [47]. The lack of the thermal energy required for the reaction, usually supplied by refluxing the reagent mixtures in

solution, has been overcome by applying neat conditions, along with the microwave assisted heating, to achieve shorter reactions time, higher yields and easier purifications while lowering the formation of side products [48, 49]. Various hemicyanine **78-92** derivatives have been obtained by mixing an aromatic aldehyde **93-100** with the appropriate quaternary salt in presence of a catalytic amount of piperidine (Figure 14). The hemicyanine **78-84** were prepared withing two minutes proving yields between 87-93% for aldehyde bearing electron donating groups (EDG) **78-81**, 86% for the unsubstituted **82** and moderately lower yields of 92 and 93% for compounds **83** and **84** with decorated electron-withdrawing groups (EWG). A similar trend in the reaction yield, ranging from 73 to 99%, was also depicted for the 4-linked hemicyanine series **85-92** over maximum reaction time up to five minutes with the reagent conversion improved and lowered by EDG and EWG respectively when compared to the unsubstituted product **89** [50].

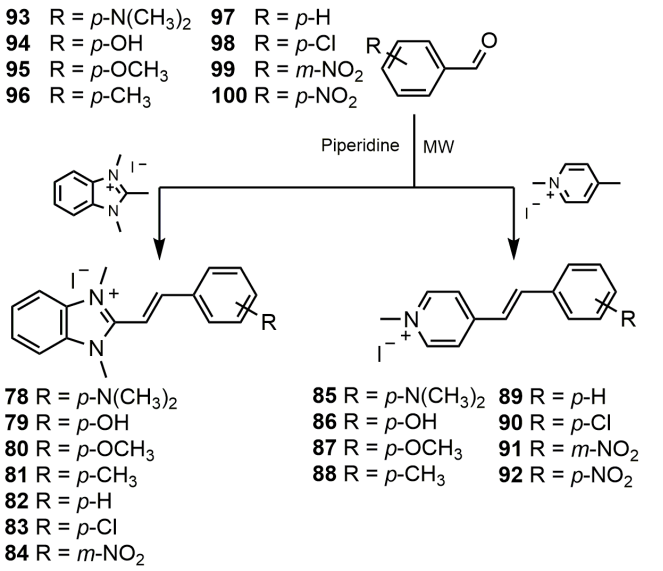

Figure 14. Hemicyanines **78-84** and **85-92** synthesized by microwave-assisted solvent-free synthesis.

A similar approach was described for the preparation of the monomethine cyanine scaffold of compound **101-104**. Neither piperidine nor pyridine alone were able to catalyze the condensation reaction between the various quaternarized precursors **105-108** and the thioether substrate **109**, most likely due to the strong and weak basicity respectively and for the rapid viscosity increase during the reaction (Figure 15a) [51]. The addition of a catalytic amount of triethylamine finally resulted in the formation of the cyanine **101-104** in high yields and short time. Dye **101**, for example was obtained in 59% yield over fifteen minutes in comparison to the reported 21% over two hours by conventional method (Figure 15b) [52]. Moreover, it is of importance to highlight that carbonization of reactive materials and products can be simply limited by lowering fine setting the microwave power (Figure 15c).

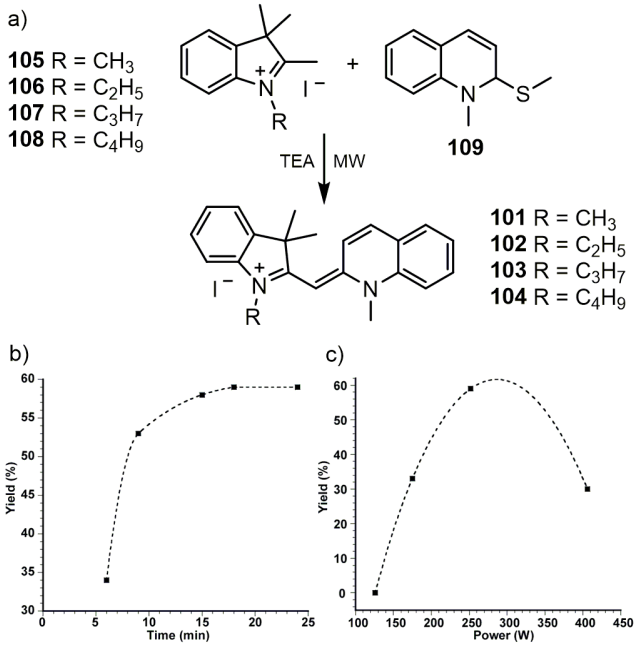

Figure 15. a) Synthetic approach to the cyanines **101-104**, b) the effect of time on yields, at a fixed power (252 W), for **101**, c) the effect of microwave power on yields of dye **101**.

A final example of the neat condition synthesis of cyanine dye **110**, whose perchlorate salt is widely used as biolabeling agent, was reported by mixing quaternary ammonium salt based on benzothiazole and the pyranone [53]. An initial grinding with a pestle, followed up but heating at 50°C for ten minutes, upon addition of a catalytic amount of perchloric acid in water afforded **110** in 30% yield while allowing the reusage of the unreacted materials (Figure 16).

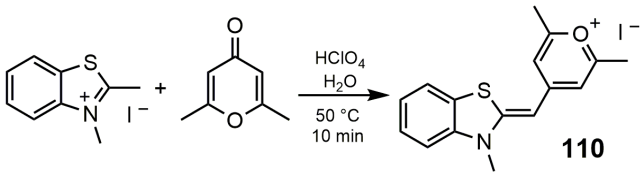

Figure 16. Synthesis of Cy39 iodide **110**.

4. CONCLUSION

Cyanines history began more than a century ago and, over the years, have been tested and used in several research fields and applications. Nevertheless, the synthetic pathway and even more the purification protocols of this family of dyes have slowed down if not hampered their preparation and availability on the market. The synthesis of asymmetrical CDs and the isolation from the symmetrical side product is nowadays challenging for organic chemists. The solid-phase syntheses methodology has been explored in the preparation of the asymmetrical CDs, at first, on the most simple and small derivatives and lately allowing the preparation of water soluble heptamethine analogs. As a general consideration, these approaches allow synthesis with higher yield alongside higher purity rate of the final compound compared to the classical approaches. Additionally, the main advantage resides on the very easy purification procedures required to isolate the desired product from impurities with high structural similarity. It is easy to speculate that synthetic procedures which lower the

consumption of organic solvents, the amount of waste while improving the product yields and reducing the reaction time will gain more and more importance in the next decades supported by the guideline of a more sustainable and environmental friendly economy and life.

CONFLICT OF INTEREST

The author(s) confirm that this article has no conflict of interest.

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